Effects of chemical seed treatment upon seed germination and stand establishment of forage crops and also upon nodulation of leguminous plants
by Daniel A Niffenegger

A THESIS Submitted to the Graduate Faculty in partial fulfillment of the requirements for the degree of Master of Science in Agronomy
Montana State University

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Three seed treating compounds—Arasan, Spergon, and Pittsburgh B-856—were compared in field and laboratory tests for the treatment of forage crops grown in Montana. Seedling emergence of alfalfa and crested wheatgrass was increased more by treatment with Arasan and Pittsburgh B-856 than by Spergon treatment;when rate of treatment was the same for all three chemicals. Response to treatment was greatest in cold, wet soil. An indication was found that excess Spergon may reduce blotter test germination results of certain crops. A corresponding reduction of germination of excess Spergon-treated seed in soil tests was not detected. Spergon adhered to seeds better than Pittsburgh B-856, and Pittsburgh B-856 adhered better than Arasan when an excess of chemical was applied. The ratio was approximately 5 : 3 : 1. Seed treatment with the three chemicals applied 1 year ahead of planting did not lower seed viability or reduce fungicidal effectiveness. Nodulation was the same on roots of alfalfa and birdsfoot trefoil when seed was both chemically treated and Rhizobium inoculated as when it was inoculated but not treated. In agar colony count tests, Pittsburgh B-856 was the most toxic to birdsfoot trefoil Rhizobium, and Spergon was the least toxic. The crop previously grown in soil appeared to affect the emergence of alfalfa and crested wheatgrass. Temperature and moisture level influenced the apparent effect of previous cropping. Chemical seed treatment increased emergence of alfalfa in all soils but did not overcome the apparent effect of previous cropping. Twenty crop species all responded favorably to seed treatment with Pittsburgh B-856 when grown in cold, wet soil.

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ABSTRACT

Three seed treating compounds--Arasan, Spergon, and Pittsburgh B-856--were compared in field and laboratory tests for the treatment of forage crops grown in Montana. Seedling emergence of alfalfa and crested wheatgrass was increased more by treatment with Arasan and Pittsburgh B-856 than by Spergon treatment when rate of treatment was the same for all three chemicals. Response to treatment was greatest in cold, wet soil. An indication was found that excess Spergon may reduce blotter test germination results of certain crops. A corresponding reduction of germination of excess Spergon-treated seed in soil tests was not detected. Spergon adhered to seeds better than Pittsburgh B-856, and Pittsburgh B-856 adhered better than Arasan when an excess of chemical was applied. The ratio was approximately 5 : 3 : 1. Seed treatment with the three chemicals applied 1 year ahead of planting did not lower seed viability or reduce fungicidal effectiveness. Nodulation was the same on roots of alfalfa and birdsfoot trefoil when seed was both chemically treated and Rhizobium inoculated as when it was inoculated but not treated. In agar colony count tests, Pittsburgh B-856 was the most toxic to birdsfoot trefoil Rhizobium, and Spergon was the least toxic. The crop previously grown in soil appeared to affect the emergence of alfalfa and crested wheatgrass. Temperature and moisture level influenced the apparent effect of previous cropping. Chemical seed treatment increased emergence of alfalfa in all soils but did not overcome the apparent effect of previous cropping. Twenty crop species all responded favorably to seed treatment with Pittsburgh B-856 when grown in cold, wet soil.

INTRODUCTION

The effects of chemical treatment of seed of forage crops on germination and stand establishment have created growing interest in recent years. Effects of treatment have ranged from decreases in stand and yield to large increases in various treatment investigations. Most of these studies have been conducted in regions having longer growing seasons, more rainfall, less severe winters, and other climatic conditions not common to Montana.

Chemically treated forage crop seed is being sold in Montana. A main selling point of one of the leading alfalfa seed brands is that it is chemically treated. General inquiries concerning forage crop seed treatment have been received at the Montana Agricultural Experiment Station. Answers to some of these inquiries are not readily available. These facts indicate a need for knowing what effects may be expected when chemically treated forage crop seed is planted in Montana.

The objectives of this study were to determine the effects of chemical seed treatment upon germination and emergence of seeds in the laboratory, upon emergence and stand establishment of forage crops in the field, and upon the nodulation of legumes.

Since limited moisture conditions are often encountered in Montana crop producing areas, it is important to know what effect chemical seed treatment may have on germination when moisture is limited. Tests on blotters and in soil were conducted in the laboratory to investigate this problem.

One of the functions of a seed laboratory is to test seed for germination. Seeding rates are based partly on the reported seed germination. Any influence that treatment may have on germination should be recognized so that seeding rates can be properly adjusted.

Emergence of chemically treated seed was studied in field and laboratory soil tests. The final indication of the value of treating seed to be planted in the field will be in the form of benefits observed under field conditions. But, since temperature and moisture conditions cannot be controlled in the field, it might take many years of field testing to draw any definite conclusions. Therefore, laboratory soil tests were made under controlled moisture and temperature conditions to supplement field tests.

Instances have been observed where alfalfa seeded in alfalfa stubble failed to produce a stand. Other crops following specific crops may show the same detrimental response. In this study, field and laboratory soil tests were conducted to find more evidence of the nature of this effect and to determine if chemical seed treatment will correct the situation.

Some legume seed must be inoculated with Rhizobium bacteria if nodules are to form on roots. Chemical seed treatment kills disease organisms living close to the seed. The question arises as to whether the chemical will also kill bacteria inoculated on the seed, leading to insufficient nodulation. This problem was investigated in field experiments with alfalfa and birdsfoot trefoil. Field tests were supplemented by laboratory agar plate tests, placing Rhizobium and chemical in contact in approximately the same ratio they would be if both were applied to seed at rates recommended by the manufacturers.

Many different crop species are grown in Montana. Many different seed treating chemicals are available. To determine the effect of different chemicals on different crop species, the effect of chemical treatment with 3 fungicides on 20 different crop species was investigated in the field and laboratory.

LITERATURE REVIEW

Chemical seed treatment of small grains has become almost standard procedure in Montana. The extent of its acceptance is evidenced by the fact that over 97 percent of all small grain samples collected in a 1956 drill box survey (16)[1] were chemically treated.

In recent years there has been growing interest in chemical seed treatment of grasses and small-seeded legumes. Results of tests conducted have been contradictory. Kreitlow et al. (10) found response from chemically treated seed of alfalfa and red clover to vary from decreases to highly significant increases in stand. Raeder (13) tested several species of forage grasses and small-seeded legumes treated with Spergon, Arasan, and New Improved Ceresan. He found no benefit from treatment. His data also showed that treating seed of low germinating capacity was no more beneficial than treating seed of high germinating capacity. Other workers (1, 6, 7) found only occasional benefits from treatment. In dry soil in the greenhouse, Gerdemann (6) obtained decreased emergence from chemically treated seed.

In other studies, treatment has appeared to be a profitable practice. On the basis of experimental results of tests in Minnesota, Kernkamp (9) recommended that alfalfa seed to be planted in Minnesota should be treated with Arasan or Spergon.

1/ Numbers in parentheses refer to literature cited.

He showed treatment to increase stand when seed was planted in soil infested with Rhizoctonia solani, or when mechanically injured seed was used. He also showed that response from treatment increased as planting depth increased. Vlitos and Preston (18), in Oklahoma, obtained increased stands from chemical seed treatment with certain chemicals.

The fungicidal value of chemicals has in some cases been offset by a harmful effect to the seed. Allison and Torrie (1) found that, following treatment with approximately a 1 percent dosage of Spergon, ladino clover and strawberry clover seed germination on nutrient agar was retarded but not reduced in total. Germination of seed of other legumes including alfalfa, sweetclover, red clover, and alsike clover, was not retarded. Arasan and New Improved Ceresan produced no effect on any of the legumes tested. In greenhouse tests, Spergon-treated ladino clover and strawberry clover seed germination was retarded and total emergence was decreased. The retarding action was also observed in the field. Forsyth and Schuster (5) reported formation of abnormal leaves on flax seedlings grown from Spergon-treated seed in nursery plantings. As the rate of treatment increased, frequency and degree of abnormality in leaves increased. Abnormality appeared to be outgrown and there was no effect on final growth and yield of the plants. Chilton and Garber (4) tested 17 species of forage legumes in flats of unsterilized soil. They found that severity of soil infestation had an effect on the chemical dosage necessary for maximum stands, and, in some cases, they found that the highest stands occurred where there was some stunting due to chemical toxicity.

Of interest to seedsmen is the question of how long a time may elapse between treatment of seed and planting. Kreitlow et al. (10) showed that treated alfalfa, red clover, and ladino clover seed stored 30 months under favorable conditions showed no injury from Arasan or Spergon. They did not show, however, whether the fungicide was still effective after this period of time. This aspect of the problem has been investigated in this study.

The degree of compatibility between Rhizobium legume inoculum and seed treating chemicals has been investigated by a number of workers. In 1936, Buchholtz (3) reported that nodules were found on the roots of plants of several legumes grown from both treated and inoculated seed. In 1944, Allison and Torrie (1) found no inhibition of nodulation due to chemical treatment. Kernkamp (8) showed in 1948 that soybean seed treatment with Spergon, New Improved Ceresan, or Semesan Jr. did not affect nodulation or yield. He concluded, in agreement with other workers, that nodulation is not affected by seed treatment or inoculation when seed is planted in soil where the nodule bacteria are already present. Later experiments by Kernkamp (9) showed that the same principle applied to other legumes. Vlitos and Preston (18) found no indication that chemical treatment inhibited nodule formation. In fact, they found that nodulation of alfalfa and Chinese cowpea was enhanced by Phygon or Arasan seed treatments followed by inoculation with Rhizobium. However, they observed, that nodulation on plants from treated seed occurred only on lateral roots. In contrast, nodulation on plants from untreated seed occurred on tap roots as well as lateral roots. Sherf and Reddy (17) concluded that in soils where bacteria are needed, chemical treatment should not be used. However, they believed that soybean-inoculating bacteria were plentiful in most Iowa soils.

Ruhloff and Burton (15) tested the compatibility of Rhizobium with several chemicals. They coated absorbent paper disks with seed protectant dusts, placed the disks on agar plates seeded with Rhizobium, and after 7 days measured the zones of inhibited growth surrounding the disks. They found that the inhibition zone for Arasan and Arasan SF was greater than for Spergon and Phygon. In the same study, they tested the survival of Rhizobium under simulated soil conditions when mixed with chemical in the ratio of 2 : 7. Their results indicated that if inoculated, chemically coated seeds are planted in moist soil, the number of Rhizobium which survive may be sufficient to bring about effective inoculation, but that under dryer conditions this may not be true.

In the present study the effects of chemical seed treatment upon stand establishment of forage crops have been measured. Yield determinations over a period of years would be a more direct measure of the benefit of stand differences brought about by seed treatment. However, stand counts are not without value.

In respect to cereal seed treatment, Leukel (11) pointed out that increased stands due to treatment do not always produce higher yields because of increased tillering of the remaining plants. However, he believed that if weeds accompany poor stands, treatment usually gives increased yields. Machacek et al. (12), in experiments in Canada, demonstrated that under weedy conditions small grains failed to compensate for low germination by increased tillering. The yield of grain under such conditions was related to the number of plants per plot.

Ronningen and Hess (14) concluded that stand counts of older established stands were of considerable value for determining relative performance of varieties of alfalfa in Maryland. It is possible that initial stands are directly correlated with stand as the planting ages.

MATERIALS AND METHODS

High quality, untreated seed, chosen from samples on file in the Montana Grain Inspection Laboratory, was used throughout this study.

Three fungicides were tested:

1. Arasan (50% tetramethyl thiuram disulfide).
2. Spergon (96% tetrachloro-para-benzoquinone).
3. Pittsburgh B-856 (50% 1,3-dichloro-5,5-diphenyl hydantoin).

Seed was treated by placing chemical and seed in a glass jar and shaking the jar by hand until the chemical was evenly distributed over the seed. Excess dosage rates were applied by adding an excess of chemical to the seed in a jar, shaking, and then screening off the excess. Until the fall of 1956, an excess dosage was applied to all seed being treated since other workers (1,9) were using this method. However, for laboratory soil tests made in the fall and early winter of 1956, $\frac{1}{2}$ of 1 percent by weight of chemical was applied.

Huffine silt loam soil from various previous croppings, including corn, small grain, alfalfa, grass, and fallow, was used in field and laboratory soil tests.

A. Field Tests

In all field tests, 100 seeds were planted $\frac{1}{4}$ to $\frac{1}{2}$ inch deep in rows 3 feet long, spaced 1 foot apart. Randomized block designs were used throughout except for a split plot design used in the test which compared the effect of the 3 chemicals upon emergence of 20 different crop species. Except where otherwise indicated, tests were replicated 4 times and stand counts were taken 2 to 3 weeks after planting.

Commercial Rhizobium inoculum for alfalfa and for birdsfoot trefoil was used in appropriate tests.

Seed was inoculated immediately before planting, as recommended by the inoculum manufacturer. Inoculation never preceded chemical treatment. When applied to the seed, a small amount of inoculum was placed in an envelope with the seed; the envelope was then shaken to distribute inoculum over the seed. Soil inoculation was accomplished by mixing a small amount of inoculum into the planting row.

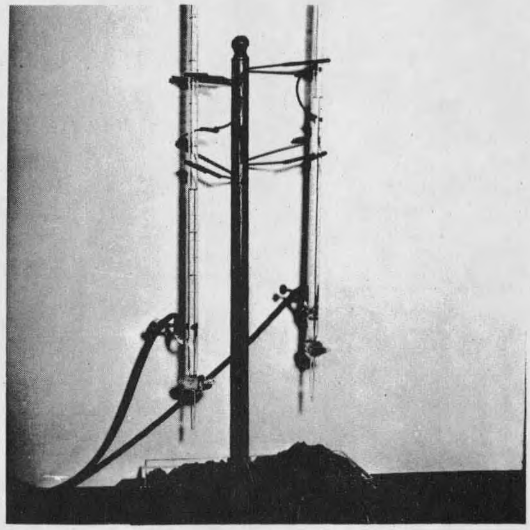
B. Laboratory Soil Tests

All laboratory soil tests were made in $1\frac{1}{2}$ x $4\frac{1}{2}$ x 5 inch plastic boxes. The equivalent of 500 grams of oven-dry soil was used in each box. Soil was sieved through a 1/12 inch round-holed sieve.

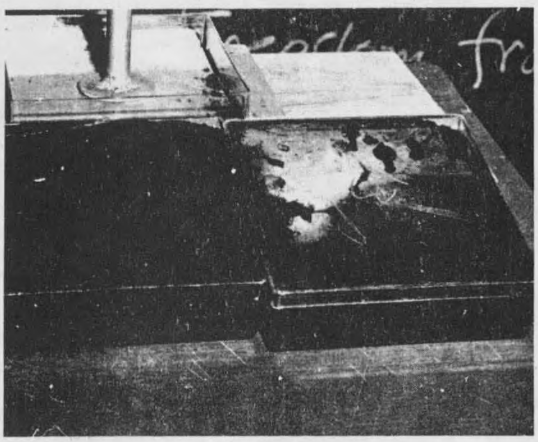
Field capacity of the soil was determined by allowing water to penetrate down into a container of air-dry soil until penetration ceased. The moisture percentage of the upper layer of soil was defined as field capacity.

Field capacity of Huffine silt loam was about 30 percent, based on oven-dry soil weight. Thirty percent was used as the wet soil moisture level. Bringing the soil to this moisture and planting the seed were done in one operation, (figure 1).

1. One hundred seeds are pressed into surface of 1/5 of soil.



2. About half of the remaining soil is added.
3. Water is added to bring to field capacity.



4. Remainder of soil is placed in box, leveled, and pressed.
5. Box is covered to reduce water loss by evaporation.

Figure 1. Method of planting laboratory soil tests at field capacity.

Dry soil tests were made in soil at 15 percent moisture. Soil moisture was raised to 15 percent by adding a calculated amount of water to the soil. After water was added, the soil was allowed to stand, except for occasional sieving, for at least 24 hours before seeds were planted. When necessary, soil was dried in a crop hot air dryer. Soil moisture determinations made after planting showed "dry" soil moisture content to range from 14-16 percent, close to the desired 15 percent.

One hundred seeds were planted in each box. Seeds were planted on top of one-fifth of the soil and covered by the other four-fifths. This gave a uniform planting depth of 1 inch. Tests were made in standard seed germinators. Temperature was controlled as follows:

2 days at 20° C.

10 days at 10° C.

10 days at 20° C.

Sprouts were counted after the 10-day period at 10° C.

The steps taken to plant seed in wet soil are shown in figure 1. Planting in dry soil was done in the same manner except that steps 2 and 3 were omitted.

C. Laboratory Blotter Tests

Standard laboratory germination tests were made in accordance with the Rules for Testing Seeds of the Association of Official Seed Analysts (2). Four hundred seeds of each lot were tested in 4 replicates of 100 seeds. Legumes were planted between water-moistened blotters and germinated at a constant 20° C. Grasses were planted on top of blotters moistened with a 0.2 percent solution of KNO_3, prechilled at 5° C. for 5 days, and germinated at an alternating temperature of 20-30° C. Normal and abnormal sprouts and hard seeds were classified as directed in the Rules for Testing Seeds (2).

Controlled moisture blotter tests were made in $1\frac{1}{2}$ x $4\frac{1}{2}$ x 5 inch plastic boxes. A single blotter, 4 x $4\frac{1}{2}$ inches, was placed in the box, and measured amounts of water were then added with a burette. A 2-day interval between adding the water and planting the seed was allowed so that the water moistened the entire blotter. All boxes were covered to reduce evaporation.

D. Laboratory Agar Plate Tests

A laboratory test of Rhizobium-chemical compatibility was made to supplement field tests of compatibility. A young, active culture of birdsfoot trefoil Rhizobium bacteria was tested for compatibility with each of the three chemicals. To insure contact of Rhizobium and chemical, measured quantities of both were placed in 500 milliliters of water and mixed in a Waring Blender for 20 seconds. A 2 : 7

Rhizobium : Chemical ratio was used since Ruhloff and Burton (15) had chosen this ratio. The mixture was immediately moved from the Waring Blender to an automatic shaker to keep the bacteria and chemical in contact. At intervals of 1½, 5, 10, and 15 minutes, transfers were made with a pipette from the mixture into distilled water. Dilutions of 1 : 100, 1 : 1000, 1 : 10,000, and 1 : 100,000 were made. A check, containing no chemical, was used through the entire procedure.

One milliliter of solution from each dilution of each treatment was transferred into a sterile petri dish. Melted mannitol agar was then added. The dish was swirled and allowed to stand for 3 days, at which time colony counts were made.

RESULTS

Comparison of Chemicals

The three chemical seed treating compounds were compared in field and laboratory trials. Table I brings together the results of chemical comparisons ignoring any interactions that may have occurred in the individual tests.

Field comparisons revealed no significant differences between the three chemicals, with one exception. In test 2, with alfalfa, Arasan treatment significantly lowered emergence. In none of the other field tests were differences between chemically treated and untreated seed significant.

In laboratory soil tests Pittsburgh B-856 appeared to be the best fungicide for alfalfa and Arasan the best for crested wheatgrass. The higher emergence of alfalfa seed treated with Pittsburgh B-856 in tests 8 and 9 was significant when compared with emergence of seed treated with Arasan. There was no significant difference between Arasan and Spergon treatments in these tests or between any of the chemical treatments in the other laboratory soil tests. The untreated check was significantly lower than the 3 chemical treatments in all laboratory soil tests except 2 (crested wheatgrass, tests 7 and 9). In test 7, the untreated check was significantly lower than Arasan and Pittsburgh B-856, but was not significantly different from Spergon treatment (Table I).

In every test on blotters excess Spergon treatment lowered germination. This was true in standard germination tests (test 12) as well as in controlled moisture blotters tests (tests 10 and 11).

Table I. Effect of chemical seed treatment on germination, emergence, and stand establishment.

Test No.	Crop	Number of samples[1]	Seed treating compound: Arasan	Spergon	Pittsburgh B-856	Untreated
		Field tests--percent emergence				
1	Alfalfa	16-16-16- 8[3]	32	33	35	33
2	Alfalfa	12-12-12- 4	32*	37	43	41
3	Crested wheatgrass	8- 8- 8- 8	18	15	21	16
4	Birdsfoot trefoil[2]	12-12-12-12	9	8	8	9
5	10 legumes	40-40-40-40	11	9	10	11
5	10 grasses	40-40-40-40	20	16	15	18
		Laboratory soil tests--percent emergence				
6	Alfalfa	6- 6- 6- 2	25*	20*	25*	5
7	Crested wheatgrass	4- 4- 4- 2	79*	65	75*	55
8	Alfalfa	20-20-20-20	50*	45*	62*	27
9	Alfalfa	20-20-20-20	30*	26*	34*	19
9	Crested wheatgrass	20-20-20-20	68	65	64	62
		Germination on blotters				
10	4 legumes	48-48-48-24	86	72*	85	86
11	4 legumes and 1 grass	40-40-40-20	79	63*	68	72
12	10 legumes	40-40-40-40	66	52*	65	65
12	10 grasses	40-40-40-40	80	77*	79	80

*Significantly different from the untreated check at 5% level.

1/ Number of samples or replications used to establish the means for each seed treatment, Arasan, Spergon, Pittsburgh B-856 and untreated respectively.

2/ Percent stand establishment.

3/ A 1955 field test. All other field tests were made in 1956.

The Effect of Excess Chemical Treatment

The lowered germination of Spergon-treated seed in laboratory blotter tests (Table I) led to an investigation to determine if differences in germination were due to differences in chemical toxicity or to differences in the amount of chemical sticking to the seed. There was evidence that more than the recommended $\frac{1}{2}$ percent rate might be sticking when chemicals were applied in excess and that the chemicals differed in sticking capacity.

To determine how much chemical was adhering to seed of different crops, 4 lots of each of 6 crops were treated with an excess of each of the 3 chemicals. Each treatment was replicated three times. The percent of chemical adhering to the seed was then determined. Treated seed weight minus untreated seed weight divided by the untreated seed weight equals the percent of chemical adhering.

The analysis of variance table of this experiment is shown in Table II and the pertinent means are shown in Table III.

More chemical adhered to seed of the grasses than to seed of legumes. The least amount of chemical adhered to regularly shaped, smooth seed of birdsfoot trefoil. Nearly five times as much Spergon as Arasan adhered to seed (Table III).

Different lots of seed of timothy, crested wheatgrass, ladino clover, and birdsfoot trefoil retained different quantities of chemical. The different lots of alsike clover and crested wheatgrass seed varied in the amounts of different chemicals they retained (Table II). In

Table II. Analysis of variance of percent chemical adhering to seed when applied in excess, based on untreated seed weight, (test 15).

Source of Variation	Degrees of Freedom	Mean Square
Crops	5	36.3453**
Chemicals	2	378.9252**
Crops x Chemicals	10	6.3242**
Lots within crops	18	5.5846**
Alfalfa lots	3	.6544
Alsike clover lots	3	2.3877
Timothy lots	3	3.6803*
Crested wheatgrass lots	3	11.6180**
Ladino clover lots	3	11.3752**
Birdsfoot trefoil lots	3	3.7921*
Lots within crops x chemicals	36	1.9604*
Alfalfa lots x chemicals	6	.7532
Alsike clover lots x chemicals	6	3.2716*
Timothy lots x chemicals	6	.5047
Crested wheatgrass lots x chemicals	6	3.4425**
Ladino clover lots x chemicals	6	2.0194
Birdsfoot trefoil lots x chemicals	6	1.7710
Error	144	1.1341

* Significant at 5% level.
** Significant at 1% level.

Table III. Mean percent chemical adhering to seed when applied in excess, based on untreated seed weight (test 15).

Crop	Seed lot	Chemical: Arasan	Chemical: Spergon	Chemical: Pittsburgh B-856	Crop Average
Alfalfa	1	0.7	4.2	1.9	
	2	0.1	5.9	2.3	
	3	0.9	5.4	2.0	
	4	0.5	4.8	2.6	
Average	of 4 lots	0.6	5.1	2.2	2.63
Alsike clover	1	1.3	6.0	2.8	
	2	1.2	8.8	2.7	
	3	0.6	5.6	2.8	
	4	1.0	5.6	4.2	
Average	of 4 lots	1.0	6.5	3.1	3.55
Timothy	1	2.1	6.6	5.2	
	2	2.1	5.5	4.7	
	3	1.5	4.2	3.7	
	4	2.4	5.6	5.3	
Average	of 4 lots	2.0	5.5	4.8	4.08
Crested wheatgrass	1	2.0	10.2	5.1	
	2	1.6	5.2	2.6	
	3	2.4	8.7	4.1	
	4	1.8	8.5	5.4	
Average	of 4 lots	1.8	8.2	4.3	4.80
Ladino clover	1	0.9	4.6	1.7	
	2	0.8	4.1	1.5	
	3	1.0	6.4	2.1	
	4	1.4	8.0	4.5	
Average	of 4 lots	1.0	5.8	2.4	3.07
Birdsfoot trefoil	1	0.8	3.8	1.7	
	2	0.2	1.9	0.9	
	3	1.2	3.8	2.4	
	4	0.4	5.3	1.6	
Average	of 4 lots	0.7	3.7	1.7	2.02
Chemical average		1.21	5.78	3.08	

general these differences were small as compared with the differences in chemicals and in species.

Treatment percentage ranged from 1 percent to 10.2 percent. It appears that the amount of chemical adhering when an excess of chemical is applied to seed will depend upon the kind of chemical being applied, the crop, the particular lot of seed of that crop being treated, and perhaps other factors.

The amount of water available to treated seed being tested in laboratory blotter tests may affect the degree of chemical concentration immediately around the seed. A higher concentration may increase chemical toxicity. To test the effect of moisture level on germination of treated seed, the critical moisture level for germination of crops to be tested was found. Four untreated seed lots of each of 6 crops were planted in controlled moisture blotter tests. Significant differences were found to exist between germination percentages at different moisture levels (Table IV). Lots within alfalfa and crested wheatgrass showed different moisture responses, but there were no significant differences between moisture responses of lots within the other crops (Table V).

Based on these results, 6 milliliters of water was used as the moisture level for testing the effect of moisture on germination of treated seed (tests 10 and 11, Table VI)[1/].

In test 11, alfalfa, alsike clover, ladino clover, birdsfoot trefoil, and timothy were tested. Timothy was not included in test 10.

Table IV. Mean germination percentage of 3 replications of 4 lots of 6 crops tested on blotters at 5 moisture levels (test 14).

Crop	Milliliters of water added to blotters				
	7 ml.	6 ml.	5 ml.	4 ml.	3 ml.
Ladino clover	83	85	78	60	4
Alfalfa	88	84	77	58	2
Alsike clover	87	86	81	54	7
Birdsfoot trefoil	60	66	46	27	4
Crested wheatgrass	87	84	33	12	0
Timothy	86	79	74	0	0
Average	82	81	54	35	3

Table V. Analysis of variance of germination percentage of 3 replications of 4 lots of 6 crops tested on blotters at 5 moisture levels (test 14).

Source of Variation	Degrees of Freedom	Mean Square
Crop	5	9,731.071**
Moisture level	4	79,374.452**
Crop x moisture level	20	2,783.686**
Lots within crops	18	929.585**
Alfalfa lots	3	1,908.333**
Alsike lots	3	544.923**
Ladino lots	3	288.411*
Timothy lots	3	289.305*
Crested wheatgrass lots	3	1,598.978**
Birdsfoot trefoil lots	3	947.528**
Lots within crops x moisture level	72	138.393
Alfalfa lots x moisture level	12	202.520**
Alsike lots x moisture level	12	130.366
Ladino lots x moisture level	12	55.383
Timothy lots x moisture level	12	101.861
Crested wheatgrass lots x moisture level	12	215.102**
Birdsfoot trefoil lots x moisture level	12	125.125
Error	240	83.647

* Significant at 5% level.
** Significant at 1% level.

Two rates of treatment and 2 moisture levels were used in both tests.

Tests 10 and 11 were conducted in the critical moisture range for germination. This is evidenced by significant differences in germination due to moisture level (Table VII). The depressing effect of excess Spergon was not affected by moisture level. However, the depressing effect of the recommended dosage rate of Spergon appeared to be overcome at a high moisture level. Further testing of this point might be of value.

Laboratory soil tests comparing the effect of moisture level on emergence of seed treated with recommended dosages of chemical were also conducted. Mean results are presented in Table VIII and the analyses of variance in Tables IX and XVII. It will be noted that the mean squares for chemical seed treatment and for chemical seed treatment by moisture level interaction are significant for both tests. Chemical seed treatment differences in dry soil were small, although the decreased emergence of Pittsburgh B-856 in test 9 was significant. In wet soil all chemical seed treatment means were significantly higher than the untreated check. Pittsburgh B-856 and Arasan treatments gave significantly higher emergence than Spergon.

1/ Seven milliliters of water absorbed by a 4 x $4\frac{1}{2}$ inch blotter approximates optimum moisture level for the standard germination procedure.

Table VI. The mean percent germination of 4 and 5 crops respectively in tests 10 and 11 when treated at 2 rates of chemical and germinated on blotters at 2 moisture levels.

Seed Treatment Chemical	Rate of Treatment	Milliliters of water applied to blotters			
		Test 10		Test 11	
		13 ml.	6 ml.	13 ml.	6 ml.
		Germination percentage			
Arasan	.5%	86	86	88	64
Arasan	Excess	87	85	87	75
Spergon	.5%	82	69	80	52
Spergon	Excess	72	64	65	54
Pitt. B-856	.5%	86	83	84	53
Pitt. B-856	Excess	88	85	83	52
Untreated	None	86	86	82	62

Table VII. Analysis of variance of tests of chemically treated seed, conducted in the critical moisture range for germination (tests 10 and 11)1/.

Source of Variation	Test 10		Test 11	
	Degrees of Freedom	Mean Square	Degrees of Freedom	Mean Square
Crop	3	6,368.40**	4	11,540.66**
Chemical seed treatment	6	1,256.35**	6	993.15**
Moisture level	1	676.01**	1	17,094.35**
Crop x moisture level	3	153.67	4	1,167.80**
Crop x Chemical seed treatment	18	488.32**	24	589.21**
Moisture level x chemical seed treatment	6	119.88	6	361.12
Crop x moisture level x chemical seed treatment	18	75.10	24	253.60
Error	112	93.38	70	277.36

** Significant at 1% level.

1/ For the purpose of analysis, each of the 2 rates of each chemical and the untreated check were considered as individual treatments.

Table VIII. Average percent emergence of chemically treated and untreated alfalfa and crested wheatgrass, planted in wet and dry soil in the laboratory (tests 9 and 13).

Chemical seed Treatment	Soil moisture level 29-31% Moisture	14-16% Moisture
Test 9 1/		
Arasan	63**	35
Spergon	55**	36
Pitt. B-856	68**	29*
Untreated	47	33
Test 13 2/		
Pitt. B-856	62**	13
Untreated	27**	9

* Significantly different from the untreated check at the 5% level.

** Significantly different from the untreated check at the 1% level.

1/ Each value is an average for 2 samples and 5 soils with different cropping histories, for both alfalfa and crested wheatgrass, or a total of 20 individual samples.

2/ Each value is an average for 4 samples and 5 soils with different cropping histories for alfalfa only, or a total of 20 individual samples.

Table IX. Analysis of variance of laboratory soil test of alfalfa and crested wheatgrass seedling emergence (test 9).

Source of Variation	Degrees of Freedom	Mean Square
Previous crop on soil	4	804.38**
Chemical seed treatment	3	702.84**
Moisture level	1	24,527.26**
Crops	1	56,137.56**
Previous crop on soil x crop	4	326.67**
Previous crop on soil x moisture level	4	249.05**
Chemical seed treatment x moisture level	3	1,134.01**
Chemical seed treatment x crop	3	301.21**
Moisture level x crop	1	3,141.76**
Previous crop on soil x chemical seed treatment	12	72.54
Previous crop on soil x chemical seed treatment x moisture level	12	129.89
Previous crop on soil x chemical seed treatment x crop	12	107.94
Previous crop on soil x moisture level x crop	4	67.15
Chemical seed treatment x moisture level x crop	3	73.71
Previous crop on soil x chemical seed treatment x moisture level x crop	12	155.26
Error	80	81.74

** Significant at 1% level.

The depressing effect of Spergon on germination in blotter tests was not evident in soil tests. This leaves results of standard germination tests of Spergon-treated seed open to question, since the germination of seed on blotters is used as the basis for determining seeding rate.

In Montana, grass and legume seeds are often exposed to cold, wet conditions, especially when they are planted early. These data indicate that treatment under cold, wet conditions is of more value than treatment under cold, dry conditions.

Time of Treatment

The length of time elapsing between seed treatment and planting was investigated from two points of view:

1. The effect of time on seed viability.
2. The effect of time on the fungicidal value of the chemicals.

Seed viability, as measured by standard germination tests of treated alfalfa seed, was not significantly different from viability of untreated seed after the seed was stored in the laboratory for 1 year (Table X).

The analysis of variance given in Table X showed no significant differences due to length of time of treatment before planting. The chemicals were similar in this regard. It may be concluded that the fungicidal value of the three chemicals was not affected by time of treatment. This indicates that there is no problem associated with storing seed treated with Arasan, Spergon, or Pittsburgh B-856.

Compatibility of Chemical Fungicides with Rhizobium

Field tests of alfalfa and birdsfoot trefoil indicated apparent compatibility between Rhizobium nodule-forming bacteria and the chemical fungicides. Mean differences in emergence of Rhizobium inoculated and uninoculated alfalfa grown in 1955 were not significant (Table XI). Examination of plant roots showed abundant nodulation regardless of inoculation or treatment. It is possible that alfalfa Rhizobium were plentiful in the soil. For this reason birdsfoot trefoil was used to test compatibility in 1956. Birdsfoot trefoil is relatively new to this region and the species of Rhizobium which will form root nodules on it must be provided in the form of commercial inoculum if nodulation is to take place. Mean results of data collected in this experiment are shown in Table XII and the analysis of variance is presented in Table XIII.

There were no significant differences in field stands $2\frac{1}{2}$ months after planting due to treatment or to inoculation (Table XIII).

Inoculation on seed or in soil significantly increased the percent of plants in flower $2\frac{1}{2}$ months after planting. Chemical seed treatment means were not significantly different. Pittsburgh B-856, the chemical seed treatment giving the highest percent of plants in flower when present on uninoculated seed or seed planted in inoculated soil, resulted in the lowest percent of plants in flower when seed was inoculated with

Table X. Effect of length of time elapsing between chemical seed treatment and planting on germination and emergence.

Test No.	Crop and Year	Time of Treatment[1]	No. of Replications	Chemical seed treatment: Arasan	Spergon	Pitt. B-856	Untreated
			Percent emergence in field tests				
1	Alfalfa 1955		4				
		2		30	33	36	
		0		34	34	34	33
2	Alfalfa 1956		4				
		12		37	35	43	
		1		24	41	43	
		0		36	36	44	41
3	Crested wheatgrass 1956		4				
		2		18	14	21	
		0		17	15	21	16
			Percent emergence in laboratory soil tests				
6	Alfalfa 1956		2				
		18		31	33	22	
		6		26	13	38	
		0		19	14	17	5
7	Crested wheatgrass 1956		2				
		2		18	14	21	
		0		17	15	21	16
			Percent germination in blotter tests				
13	Alfalfa 1956		4				
		12		74	73	75	75
14	Crested wheatgrass 1957		4				
		5		85	93	91	92
15	Alfalfa 1957		4				
		5		86	81	79	85

[1] Number of months elapsing between seed treatment and planting.

Table XI. The effect of inoculation with Rhizobium of chemically treated seed on the emergence of alfalfa in 1955 field tests (test 1)1/.

Rhizobium Inoculation	Chemical seed treatment			
	Arasan	Spergon	Pitt. B-856	Un-treated
Inoculated on seed	32	34	34	34
Not inoculated	32	33	37	32

1/ Values represent average percent emergence of 4 replications.

Table XII. Mean values for birdsfoot trefoil establishment, maturity, and nodulation in field tests of inoculated and chemically treated seed (test 4).

Method of inoculating with Rhizobium	Seed treatment: Arasan	Spergon	Pitt. B-856	Untreated	Average
	Percent of seed planted establishing plants				
On seed	6	8	11	9	9
In soil	12	8	7	12	10
None	11	6	8	5	7
Average	9	8	8	9	
	Percent of plants in flower				
On seed	46	53	27	32	40*
In soil	48	15	54	35	38*
None	21	5	50	22	22
Average	39	21	44	26	
	Nodulation score				
On seed	4.12	3.11	2.89	3.41	3.38**
In soil	3.68	3.31	3.58	3.22	3.45**
None	1.37	1.53	2.35	1.93	1.79
Average	3.06	2.65	2.94	2.85	

* Significantly higher than the uninoculated check at the 5% level.
** Significantly higher than the uninoculated check at the 1% level.

Table XIII. Analysis of variance of the effect of Rhizobium inoculation and chemical seed treatment on field stand, nodulation score, and percent plants in flower of birdsfoot trefoil (test 4).

Source of Variation	Degrees of Freedom	Mean squares: Field Stand	Mean squares: Nodulation Score	Mean squares: Percent plants in flower
Replication	3	79.3*	1.01	137.9
Chemical seed treatment	3	8.0	0.35	1,113.8
Rhizobium inoculation	2	19.0	14.02**	1,604.7*
Inoculation x chemical seed treatment	6	27.7	0.87	1,132.2*
Error	33	25.6	0.48	413.8

* Significant at 5% level.
** Significant at 1% level.

Rhizobium. This indicates an effective fungicidal action of the chemical, but a lack of compatibility of this chemical and Rhizobium. Results with Spergon were the opposite, indicating poor fungicidal action but good compatibility with Rhizobium (Table XII).

Roots of the birdsfoot trefoil plants were examined for nodules. A system of scoring plants for nodulation was established. Scoring was based on the relative number of nodules per individual plant. A zero score was given when no nodules were present and a score of 5 was given to plants possessing large numbers of nodules. Inoculation appeared to be essential for a high nodulation score, but method of inoculating was of no consequence. Differences due to chemical seed treatment and to chemical seed treatment by Rhizobium inoculation interaction were not significant.

The toxicity of seed treatment compounds to Rhizobium was measured further by agar plate tests. Colony counts at 1 : 100 dilution were found to be best for making comparisons. Pittsburgh B-856 was the most toxic to birdsfoot trefoil Rhizobium. Spergon was the least toxic but still killed large numbers of bacteria when compared with the untreated check. Differences due to time of contact were not significant but chemical by time interaction was significant at the 5 percent level. This is due to killing of bacteria by time of contact with Arasan and Pittsburgh B-856, but not with Spergon (Table XIV).

Table XIV. Colony counts of Rhizobium on agar plates when exposed for varying periods of time to 3 chemicals and a control treatment.

Time of contact with chemical	Chemical seed treatment			
	Arasan	Spergon	Pitt.B-856	Untreated
$1\frac{1}{2}$ minutes	103	170	29	400 plus
5 minutes	121	142	29	400 plus
10 minutes	74	139	18	550
15 minutes	21	184	12	400 plus
Chemical mean values	80**	159**	20	450 plus

** Significantly higher than Pitt.B-856 at 1% level. All chemical mean values were significantly lower than the untreated check.